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Faculty of Pharmacy
2, Dunav str., Sofia 1000
Fax (02) 987 987 4

Editor in Chief: ✆(+359 2) 9236 505
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CONTENTS

ANTIMYCOBACTERIAL ACTIVITY OF NOVEL CAMPHANE BASED ISOINDOLINE

G. Stavrakov[1], V. Valcheva[2], G. Dobrikov[3]

[1]Faculty of Pharmacy, Medical University of Sofia, str. Dunav 2, Sofia 1000, Bulgaria
[2]Institute of Microbiology, Bulgarian Academy of Sciences, Akad. Bonchev 26, Sofia 1113, Bulgaria
[3]Institute of Organic Chemistry, Bulgarian Academy of Sciences, Acad. Bonchev 9, Sofia 1113, Bulgaria

Summary: A new isoindoline containing amino-alcohol was synthesised on the base of the camphor scaffold and evaluated for its in vitro activity against *M. tuberculosis* $H_{37}Rv$. The compounds shows activity comparable with the one of the classical anti-TB drug ethambutol. The camphane-based structures present novel promising scaffolds for antimycobacterial agents.

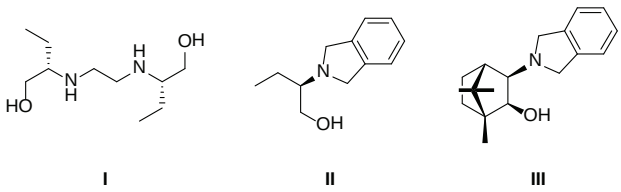
Key Words: camphane, isoindoline, antimycobacterial activity, *M. tuberculosis* $H_{37}Rv$

Introduction

Tuberculosis (TB) is a global health problem causing substantial morbidity, mortality, negative socioeconomic impact, and human suffering. One-third of the world's population is latently infected with *Mycobacterium tuberculosis* and approximately 9 million cases of active disease occur each year [1]. The recent widespread emergence of multidrug resistant (MDR) strains of *M. tuberculosis* to clinically available drugs puts further impetus to the urgent need for the discovery of new and effective anti-TB agents. Much progress has been done in drug development over the past decade. Currently, there are at least nine compounds in clinical development: two in phase III, four in phase II, and three in phase I trials [2]. Among these, four are existing drugs redeveloped for a TB indication and five are new chemical entities. More than 30 new anti-TB drugs are in preclinical development [3,4].

Wilkinson and coworkers first reported the synthesis and activity of ethambutol (EMB) (Fig. 1. **I**) [5]. EMB was a useful addition to tuberculosis chemotherapy, despite a relatively modest MIC of 10 μM, in part because of very low toxicity and relatively few side-effects. Based on structure-activity relationship (SAR) studies it appeared that the distance between the two nitrogens, the presence of β-aminoalcohols, and the small side chains were critical for determining activity [6].

Inspired by the two β-aminoalcohol fragments in the molecule of EMB we dedicated our studies towards the development of mono-aminoalcohols bearing different pharmacophore fragments and evaluation of their antimycobacterial activity [7]. Most of the compounds containing the (S)-2-amino-1-butanol motif are showing similar but not significantly higher activity than EMB. For example, the isoindoline containing structure (Fig. 1. **II**) gave MIC of 10.46 μM towards the referent strain of *M. tuberculosis* $H_{37}Rv$, which is comparable with the MIC of EMB (7.22 μM).



I II III

Figure 1.

In the present study we describe the synthesis and antimycobacterial activity of a novel anti-TB compound, containing camphane moiety. Molecules possessing bornyl fragment exhibit a variety of pharmacological activities, including antibacterial, antifungal, anti-inflammatory, and anesthetic. We were intrigued to combine the camphane scaffold with the β-aminoalcohol isoindoline containing fragment (Fig. 1. **III**) and evaluate the antimycobacterial activity towards *M. tuberculosis* $H_{37}Rv$.

Materials and Methods

1. Chemistry

Reagents were commercial grade and used without further purification. Thin layer chromatography

(TLC) was performed on aluminum sheets pre-coated with Merck Kieselgel 60 F_{254} 0.25 mm (Merck). Flash column chromatography was carried out using Silica Gel 60 230-400 mesh (Fluka). Commercially available solvents for reactions, TLC and column chromatography were used after distillation. The NMR spectra were recorded on a Bruker Avance II+ 600 spectrometer (600.13 for ^{1}H MHz, 150.92 MHz for ^{13}C NMR) with TMS as internal standards for chemical shifts (δ, ppm). ^{1}H and ^{13}C NMR data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, m = multiplet), coupling constants (Hz), integration, identification. The assignment of the ^{1}H and ^{13}C NMR spectra was made on the basis of DEPT, HSQC, and NOESY experiments. Elemental analyses were performed by Microanalytical Service Laboratory of Faculty of Pharmacy, Medical University of Sofia, using Vario EL*3* CHNS(O). Dimethyl sulfoxide (DMSO) for testing of bioactivities was commercial (spectroscopic grade) and was used without distillation.

Preparation of (1R,2S,3R,4S)-3-(isoindolin-2-yl)-1,7,7-trimethylbicyclo[2.2.1]heptan-2-ol 4:

To a stirred solution of 3-*exo*-aminoisoborneol (0.5 g, 2.95 mmol) in 15 ml CH_3CN were added α,α-dichloro-*o*-xylene (0.517 g, 2.95 mmol), K_2CO_3 (0.450 g, 3.25 mmol), and a crystal of 18-crown-6. The mixture was refluxed for 6 h and left at room temperature overnight. The CH_3CN was concentrated under reduced pressure and the residue was separated between CH_2Cl_2 and water. Extraction with CH_2Cl_2 was followed by washing with water, drying above K_2CO_3, filtration and concentration. The product was purified by flash column chromatography on silica gel (hexane/EtOAc = 9:1) to give 0.392 g (49% Yield) of **4** as white crystals; m.p. xx °C. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 0.84 (s, 3H), 1.01 (s, 3H), 1.05-1.12 (m, 2H), 1.16 (s, 3H), 145-1.53 (m, 1H), 1.74-1.80 (m, 1H), 2.06 (d, 1H, *J* = 4.7 Hz), 2.82 (d, 1H, *J* = 7.1 Hz), 3.53 (d, 1H, *J* = 7.1 Hz), 3.86 (br d, 2H, *J* = 11.1 Hz), 3.95 (br s, 1H, OH), 4.20 (br d, 2H, *J* = 11.9 Hz), 7.23 (s, 4H) ppm. ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ = 11.58, 20.86, 22.13, 27.74, 32.41, 46.34, 48.93, 49.37, 59.42 (2C), 72.57, 79.88, 122.15 (2C), 126.80 (2C) ppm. $C_{18}H_{25}NO$ (271.40) calcd. C 79.77, H 9.28, N 5.16; found C 79.41, H 9.34, N 5.37.

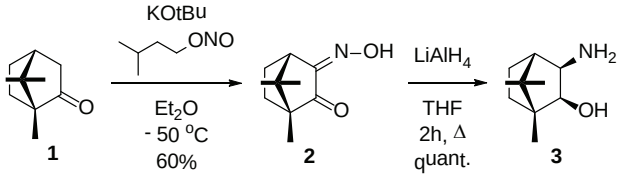
2. Antimycobacterial activity

The antimycobacterial activity was determined through the proportional method of Canetti towards reference strain *M. Tuberculosis* $H_{37}Rv$. This method, recommended by the WHO, is the most commonly used one worldwide for exploration of sensitivity/resistance of tuberculosis strains towards chemotherapeutics [8-12]. It allows precise determination of the proportion of resistant mutants to a certain drug.

A sterile suspension/solution of the tested compound was added to Löwenstein-Jensen egg based medium before its coagulation (30 min at 85 °C). The compound was tested at four concentrations – 5 mg/ml, 2 mg/ml, 0.2 mg/ml and 0.1 mg/ml (in DMSO). Tubes with Löwenstein-Jensen medium (5 ml) containing the tested compound and those without them (controls) were inoculated with a suspension of *M. tuberculosis* $H_{37}Rv$ (10^5 cells/ml) and incubated for 45 days at 37 °C. The ratio between the number of colonies of *M. tuberculosis* grown in medium containing compounds and the number of colonies in control medium were calculated and expressed as percentage of inhibition. The MIC is defined as the minimum concentration of compound required to inhibit bacterial growth completely (0% growth). The MIC values are calculated and given as μM.

Results and Discussion

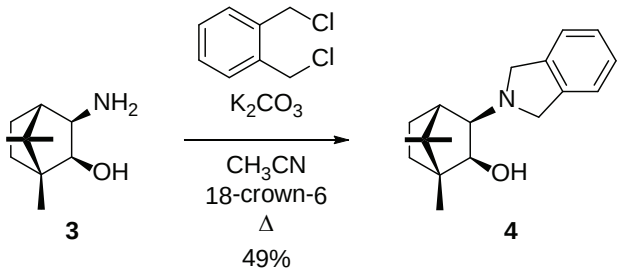
Monoterpenoids have long been widely used as chiral, enantiopure starting materials in natural product synthesis. Among the numerous monoterpenoids, the camphor derivatives are of particular importance because of their widespread occurrence in plants [13]. In spite of the fact that the chemistry of camphor is as old as the chemistry itself, this natural product and its derivatives still remain attractive as inexpensive source of enantiopure building blocks for organic synthesis [14].



Scheme 1.

Intrigued to synthesize amino-alcohols containing camphene skeleton we chose 2-(*S*)-(-)-3-*exo*-aminoisoborneol **3** as the key starting compound. The latter is readily available from (+)-camphor **1**

in two steps (Scheme 1) [15]. Deprotonation of the a-position of camphor at -50 °C with potassium *tert.*-butoxide followed by reaction with isopentyl nitrite lead to the preparation of oxime **2** in 60% yield. The following reduction with lithium aluminium hydride gave quantitatively the desired 3-*exo*-aminoisoborneol **3** (Scheme 1).



Scheme 2.

The introduction of the isoindoline fragment was accomplished by refluxing the camphor derived aminoalcohol with o-xylenedichloride in acetonitrile, and in the presence of catalytic ammounts of 18-crown-6, and potasium carbonate as base. The desired product was isolated after flash column chromatography as white crystals in 49% Yield (Scheme 2).

The synthesized compound was evaluated for its in vitro activity against *M. tuberculosis* $H_{37}Rv$ using the method of Canetti. The compound is in agreement with the formal Lipinski's rule of five. The amino-alcohol exhibited activity with MIC of 7.37 μM, which is comparable with the one of the reference compound ethambutol dihydrochloride with MIC of 7.22 μM.

Conclusion

In summary, we have synthesized a new isoindoline containing amino-alcohol on the base of 3-*exo*-aminoisoborneol. The compound was screened for antimycobacterial activity against the $H_{37}Rv$ Strain of *Mycobacterium tuberculosis*. The molecule shows activity comparable with the one of the classical anti-TB drug ethambutol. Obviously, camphane-based structures are promising scaffolds for novel antimycobacterial agents.

Acknowledgement

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